A COMPARATIVE STUDY OF SODIUM CITRATE AND TARTRATE IN THE DIFFERENTIATION OF *B. COLI* IN WATER ANALYSIS

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PLATES XXV–XXVII

Brown (1921), in a study of the use of citrated media for the cultivation of micro-organisms, showed that in a 1 per cent. sodium citrate broth certain bacteria fail to grow, and the addition to the cultures after forty-eight hours' incubation, of a saturated solution of lead acetate, produces a copious white precipitate, whilst other organisms utilise the citrate for growth and lead acetate yields only a slight precipitate.

Stewart Koser (1923), in an exhaustive study of the utilisation of salts of various organic acids by the colon aerogenes group of organisms, showed that the group is divisible into two sections, one a *B. coli*, or faecal type, which fails to utilise sodium citrate in a synthetic medium, and the other a *B. aerogenes*, or non-faecal type, which produces a visible turbidity, usually within forty-eight hours.

Brown, Duncan, and Henry (1923), in an extensive study of the fermentation of salts of organic acids as an aid to the differentiation of bacterial types, showed that with stock strains of organisms *B. lactis aerogenes* and *B. cloacae* are capable of decomposing citrate, while *B. coli communis*, *B. coli communior* and *B. acidi lactici* fail to attack citrate, and that this difference is also recognisable by the use of lead acetate in a definite quantitative relation to the medium employed.

In previous communications the results of the application of Koser's citrate medium to colon organisms isolated in Trinidad, from faeces, unpolluted soils and waters of various degrees of sanitary purity were recorded, and the suggestion made that in the bacterio-

logical analysis of unpurified and doubtful water supplies it should be employed as a means of differentiation between faecal and non-faecal coli organisms.

In the present contribution will be seen the findings of a comparative study of the results obtained with Koser's medium, and the methods recommended by Brown, Duncan, and Henry, as applied to coli organisms isolated directly from faeces and from water supplies in Trinidad.

Koser's medium was the one employed in previous studies and already described. It consisted of 1·5 grammes microcosmic salt, Na (NH_4) HPO_4, 4 aq., 1 gramme KH_2PO_4, 0·2 gramme $MgSO_4$, and 2 grammes sod. citrate in 1,000 c.c. distilled water, tubed and autoclaved at 120° C. for fifteen minutes. The cultures were incubated at 30° C. for two to three days, after which time readings were made, a clear colourless liquid indicating a faecal organism, and turbidity with a slight deposit, a non-faecal one. The medium recommended by Brown, Duncan, and Henry, consisted of 1 per cent. sod. citrate, or 1 per cent. sod. tartrate in a 1 per cent. peptone water (bacto-peptone of the Digestive Ferment Co., 1 gramme, sod. chloride 0·05 gramme, aq. distil. 100 c.c.). About 3 c.c. portions were tubed and autoclaved at 115° C. for twenty minutes. The cultures were incubated at 37° C. for forty-eight hours, and three drops of a saturated solution of lead acetate added, the tubes well shaken and examined the following day. In one series of experiments the lead acetate was added to the culture tubes in the definite proportions recommended by Brown and his associates. The formation of a voluminous white flocculent precipitate was evidence that the salt was not decomposed and the organism was of faecal origin. Should a small granular precipitate form which slowly settles to the bottom of the tube, decomposition of the salt was considered to have taken place and the organism to have been a non-faecal one.

Fifty *B. coli* colonies (lactose + indol +) were isolated from five different samples of cow dung secured from five different bovines. All these fifty colonies failed to produce turbidity in Koser's medium, in accordance with their faecal origin, except colony No. 21, which showed a faint turbidity *without deposit* in three days. In the tartrate medium all fifty colonies produced a heavy white flocculent precipitate, agreeing with their faecal source.

In a similar manner fifty colon organisms were isolated from three different samples of human faeces from three different individuals. All these colonies fermented lactose with the production of acid and gas, and yielded indol by Ehrlich's method. All failed to utilise the citrate and tartrate in the bacto-peptone media and the addition of lead acetate produced an abundant white flocculent precipitate as would be expected of faecal coli. In Koser's citrate media they also failed to produce turbidity, agreeing with the characteristic or faecal colon organisms, with the exception of one colony which produced a visible turbidity *but no deposit* in seventy-two hours. As in the previous experiments there was no quantitative relation between the lead acetate and the bacto-peptone media in the performance of these tests.

These results, though obtained with a small number of cultures, indicate that these media and methods may be of distinct value in detecting the presence of colon organisms isolated from human faeces and cow-dung, but it was necessary to submit them to the action of colon organisms isolated from water supplies to justify an expression of opinion as to their comparative value in water bacteriology.

Two samples (25 c.c. each) of water were collected from an undeniably pure source, viz., the Chorah Ravine. This stream rises from an uninhabited and wooded land, free from all possibility of human pollution and without any evidence of animal faecal contamination, to flow subterraneously for about fifty yards and escape at the side of a hillock. From this outlet the samples were taken. Forty colon colonies were obtained in the routine way and inoculation made into lactose-peptone water, Witte's peptone water, Koser citrate and citrate and tartrate bacto-peptone, and tested as above explained. All the forty colonies fermented lactose with the production of acidity and gas, all produced indol except colonies No. 7 and No. 15, and they all utilised Koser's citrate with the production of a visible turbidity indicative of a non-faecal origin. In the citrate bacto-peptone medium with the addition of lead acetate, only seventeen were found to yield a slight granular precipitate, which is considered to be evidence of a non-faecal origin, and the remaining twenty-three produced a heavy white flocculent precipitate indicative of a faecal source. Both the colonies which

failed to produce indol (i.e., Nos. 7 and 15) yielded in citrate bacto-peptone a slight granular precipitate. In the tartrate bacto-peptone all the forty colonies produced a heavy white precipitate characteristic of faecal coli.

It is thus seen that, of the three methods, Koser's correlates most accurately with the origin of the cultures when obtained from a sanitarily pure source.

Table I gives these various results.

Table I.

Koser's citrate		Bacto-peptone citrate		Bacto-peptone tartrate		No. of Colonies	Source
Pos.	Neg.	Pos.	Neg.	Pos.	Neg.		
1 ?	49	—	—	0	50	50	Cow Dung
1 ?	49	0	50	0	50	50	Human Faeces
40	0	17	23	0	40	40	Unpolluted Water

Positive = utilisation of citrate = non-faecal organism.
Negative = no utilisation of citrate = faecal organism.
It should be noted that the lead acetate was not added in measured proportion to the media.

Whilst, therefore, of one hundred colon organisms isolated from faeces, 98 per cent. were definitely faecal in their action upon Koser's citrate, and 2 per cent. may be considered doubtful; of fifty such organisms isolated from cow-dung, and fifty from human faeces, 100 per cent. were faecal in their mode of action upon bacto-peptone tartrate and citrate. On the other hand, of forty colon organisms obtained from an undeniably pure water supply, whilst 100 per cent. were non-faecal in their action upon Koser's citrate, only 42·5 per cent. can be considered as of non-faecal origin in the case of bacto-peptone citrate, and with bacto-peptone tartrate they would all be regarded as having a faecal source and the water as being grossly contaminated. Here again it cannot be too strongly emphasised that the valuation of any reaction supposedly characteristic of *B. coli* should be based upon an examination of *B. coli* as isolated not only from faeces but also from waters of various degrees of sanitary purity.

In addition, forty coli-like colonies were isolated in the height of the dry season from four different samples (25 c.c. each) of water collected from the Maraval River below the Maraval Reservoir. At the Reservoir the river water is chlorinated so as to remove lactose fermenters from 50 c.c. On 29 March, with the cleaning of the tanks, this chlorinated water was allowed to escape into the river which flows along a public road exposed to pollution from a few houses in the neighbourhood and which also receives the morning overflow. The samples were collected on 29 March, about one mile below the Reservoir. These forty colonies, all of which were lactose fermenters, and thirty-nine of which were indol producers, were subjected to the action of Koser's citrate, bacto-peptone citrate and tartrate in the manner already described, with the following results.

Table II.

Lactose		Indol		Koser's citrate		Bacto-peptone citrate		Bacto-peptone tartrate	
Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
40	0	39	1	21	19	18	22	13	27

In order, however, to correlate more closely the results of sanitary and topographical survey with the bacteriological findings, samples of water were collected from the Lopinot River at various levels, as shown on the sketch map, by skirting the bank of the river in a motor car, and 25 c.c. from each sample were inoculated into MacConkey lactose bile salt medium, double strength, plates being made on Rebipelagar and red or reddish colonies picked out on to agar slants on which they were kept for some months until ready for use, when a further subculture on agar was made and growth allowed to proceed for twenty-four hours. To follow strictly the procedure recommended by Brown and his associates, from the agar slants, inoculations were made into peptone broth and allowed to incubate for twenty-four hours at 37·5° C. From the broth tubes about 3 mm. loopfuls were added to citrate bacto-peptone water, tartrate bacto-

peptone water, Koser's citrate, Witte's peptone water and lactose-peptone water. For the sake of economy the bacto-peptone media were tubed in 2·5 c.c. quantities and after twenty-four hours' incubation at 37·5° C., 0·2 c.c. of an aqueous saturated solution of lead acetate was added to the citrate and 0·3 c.c. to the tartrate, in the proportion recommended by Brown and his co-workers. Koser's citrate tubes were read in three days' time after incubation at 30° C., and Ehrlich's Para-dimethyl-amido-benzaldehyde test for indol performed after three days' incubation at 37° C. The results are shown on sketch map (p. 311), and in Table III.

The lack of correlation between the bacteriological findings and the sanitary survey in the case of the tartrate medium, as exemplified by the results obtained with both the Chorah and Lopinot waters, particularly with the former, indicates that this medium conveys little or no information as to the degree of purity of a water supply and may be disregarded in routine bacteriological analysis. In connection with the bacto-peptone citrate and Koser's citrate, the results from the Maraval and Lopinot Rivers show that the difference between these media is small, but when the Chorah water results are examined it is seen that Koser's citrate reveals more accurately the source of the cultures.

It should, however, be remembered that it may not be possible with Koser's citrate to express an opinion as to the sanitary source of a colon organism until incubation has proceeded for more than two to three days, and there may still be an element of doubt, whilst the application of the bacto-peptone citrate medium necessitates the additional use of another reagent and more labour.

Photographs 1 to 9, illustrating the nature of the precipitate obtained with the bacto-peptone media, explain themselves. It is seen that the precipitate formed when the lead acetate solution is not added in the definite measured proportion recommended by Brown and his associates may vary considerably in quantity.

TABLE III.

Showing results of the action of coli organisms isolated from various levels of the Lopinot River Trinidad, upon sodium citrate and tartrate.

	Lactose	Indol	Koser's citrate	Bacto-peptone citrate	Bacto-peptone tartrate	
1	+	−	+	+	+	Sample taken at 8¼ miles. Upper part of River. *Little or no evidence of Pollution.*
2	+	−	+	+	−	
3	+	+	+	+	−	
4	+	+	+	+	−	
5	+	+	+	+	−	
6	+	+	+	+	−	
7	+	−	+	+	−	
8	+	−	+	+	−	
9	+	−	−	+	−	
10	+	−	+	+	−	
11	+	−	+	+	+	
12	+	−	+	+	−	
13	+	−	+	+	+	
14	+	−	+	−	−	
15	+	+	+	+	−	
16	+	+	+	+	+	
17	+	−	+	−	+	
18	+	−	+	+	+	
19	+	−	+	−	+	
20	+	−	+	+	+	
21	+	+	+	−	−	Sample taken at 7¼ miles. Lower down stream than previous. *Some Pollution.*
22	+	+	−	−	−	
23	+	+	−	−	−	
24	+	+	+	−	−	
25	+	+	−	−	−	
26	+	+	−	−	−	
27	+	+	−	−	−	
28	+	+	−	−	−	
29	+	+	−	−	−	
30	+	+	−	−	−	
31	+	+	+	−	−	
32	+	+	+	−	+	
33	+	+	+	−	−	
34	+	+	−	−	−	
35	+	+	+	−	+	
36	+	+	−	+	−	
37	+	+	−	−	−	
38	+	+	−	−	−	
39	+	+	+	+	−	
40	+	+	−	−	−	
41	+	+	−	−	−	Sample taken at 7¾ miles. *Much Pollution.*
42	+	+	−	−	−	
43	+	+	−	−	−	
44	+	+	−	−	−	
45	+	+	−	−	−	
46	+	+	−	−	−	
47	+	+	−	−	−	
48	+	+	−	−	−	
49	+	+	−	−	−	
50	+	+	−	−	−	
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54	+	−	+	+	−	
55	+	+	−	−	−	
56	+	+	−	−	−	
57	+	+	−	−	−	
58	+	+	−	−	−	
59	+	+	−	−	−	
60	+	+	−	−	−	
61	+	+	−	−	−	Sample taken at 6¼ miles. Exposure to sunlight. *No Pollution.*
62	+	−	+	+	−	
63	+	+	−	−	−	
64	+	+	−	−	−	
65	+	+	−	−	−	
66	+	+	−	−	+	
67	+	−	+	+	−	
68	+	+	−	−	−	
69	+	+	−	−	−	

TABLE III.—*continued.*

	Lactose	Indol	Koser's citrate	Bacto-peptone citrate	Bacto-peptone tartrate	
70	+	+	−	−	−	Sample taken at 6¼ miles. Exposure to sunlight. *No Pollution.*
71	+	−	+	+	+	
72	+	−	+	+	+	
73	+	−	+	+	+	
74	+	−	+	−	−	
75	+	+	−	−	−	
76	+	−	+	+	−	
77	+	+	+	+	+	
78	+	−	+	−	−	
79	+	−	+	+	−	
80	+	−	+	+	−	
81	+	+	−	−	−	Sample taken at 5¼ miles. Below village. *Gross Pollution.*
82	+	+	−	−	−	
83	+	+	−	−	−	
84	+	+	−	−	−	
85	+	+	−	−	−	
86	+	+	−	−	−	
87	+	+	−	−	−	
88	+	+	−	−	−	
89	+	+	−	−	−	
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94	+	+	−	−	−	
95	+	+	−	−	−	
96	+	+	−	−	−	
97	+	+	−	−	−	
98	+	+	−	−	−	
99	+	+	−	−	−	
100	+	+	−	−	−	
101	+	+	−	−	−	Sample taken at 1¾ miles. *Exposed to sunlight.*
102	+	+	−	−	−	
103	+	+	−	−	−	
104	+	+	−	−	−	
105	+	+	+	+	−	
106	+	−	−	−	−	
107	+	+	−	−	−	
108	+	+	−	−	−	
109	+	+	−	−	−	
110	+	+	−	−	−	
111	+	+	−	−	−	
112	+	+	−	−	−	
113	+	−	+	+	+	
114	+	+	−	−	−	
115	+	+	−	−	−	
116	+	+	−	−	−	
117	+	+	−	−	−	
118	+	+	−	−	−	
119	+	+	−	−	−	
120	+	+	+	−	−	
121	+	+	−	−	−	Sample taken at 'terminus' of River. *Occasional Pollution above.*
122	+	+	−	−	−	
123	+	+	−	−	−	
124	+	+	−	−	−	
125	+	+	−	−	−	
126	+	+	−	−	−	
127	+	+	−	−	−	
128	+	+	−	−	−	
129	+	+	−	−	−	
130	+	+	+	+	−	
131	+	+	−	−	−	
132	+	+	−	−	−	
133	+	+	−	−	−	
134	+	+	−	−	−	
135	+	+	−	−	−	
136	+	+	−	+	−	
137	+	+	−	−	−	
138	+	+	−	+	−	
139	+	+	+	+	−	
140	+	+	−	−	−	

LOPINOT RIVER, TRINIDAD.

Table illustrating the action of coli organisms isolated from various levels upon sodium citrate and tartrate.

Little or no evidence of pollution		Some pollution		Much pollution		Exposure to sunlight. No pollution		Gross pollution		Exposure to sunlight		Exposure to sunlight. Occasional pollution		Test substance
8¼ miles		7¼ miles		7¼ miles		6¼ miles		5¼ miles		1¼ miles		0 mile		
Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	
20	0	20	0	20	0	20	0	20	0	20	0	20	0	Lactose
6	14	20	0	19	1	10	10	20	0	18	2	20	0	Indol
19	1	7	13	1	19	11	9	1	19	3	17	2	18	Koser's citrate
8	12	2	18	0	20	5	15	0	20	1	19	0	20	Bacto-peptone tartrate
17	3	2	18	1	19	9	11	2	18	2	18	4	16	Bacto-peptone citrate

Pos. Koser's citrate
Pos. Bacto-peptone citrate
Pos. Bacto-peptone tartrate
} = Utilisation of citrate and tartrate = non-faecal coli organism.

Neg. Koser's citrate
Neg. Bacto-peptone citrate
Neg. Bacto-peptone tartrate
} = No utilisation of citrate and tartrate = faecal coli organism.

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EXPLANATION OF PLATE XXV

Fig. 1. No decomposition of tartrate. Measured volumes. Voluminous white flocculent precipitate; note 'bursting' of precipitate by gas.

Fig. 2. Decomposition of tartrate. Measured volumes. Small heavy granular precipitate.

Fig. 3. No decomposition of citrate. Measured volumes. Voluminous white flocculent precipitate.

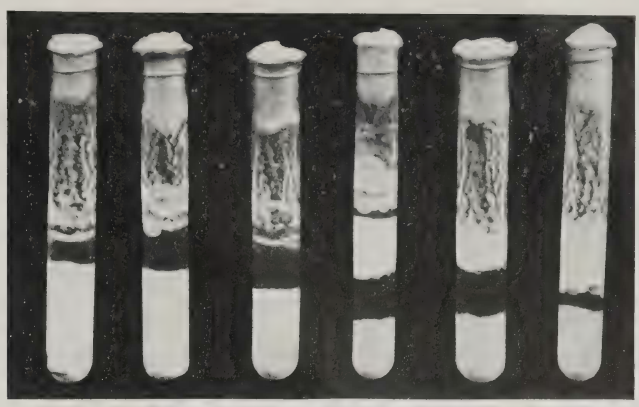

FIG. 1

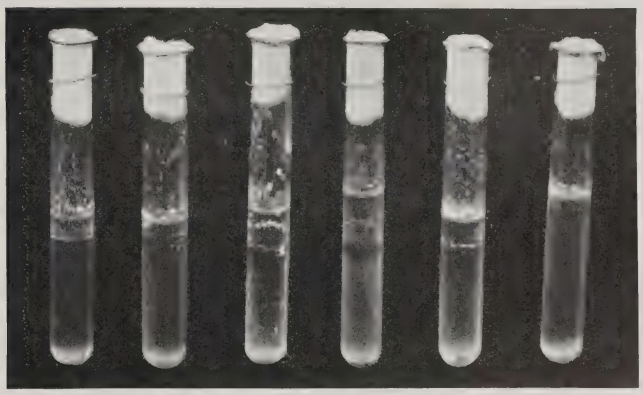

FIG. 2

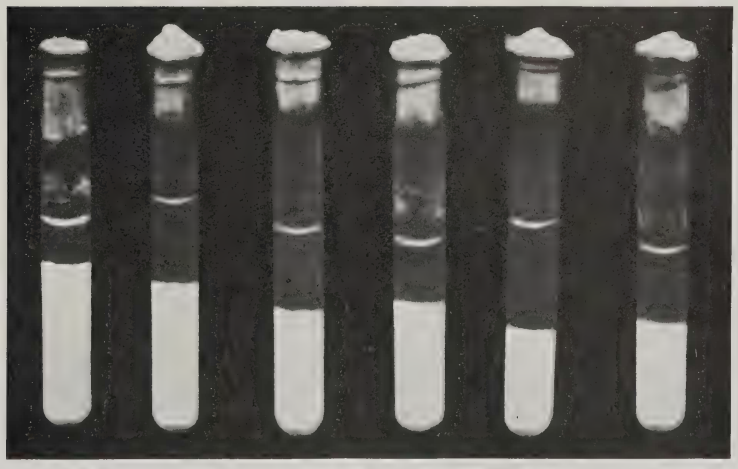

FIG. 3

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EXPLANATION OF PLATE XXVI

Fig. 4. Decomposition of citrate. Measured volumes. Small heavy granular precipitate.

Fig. 5. No decomposition of tartrate. Voluminous white flocculent precipitate formed.

Fig. 6. Decomposition of tartrate. Small heavy granular precipitate formed.

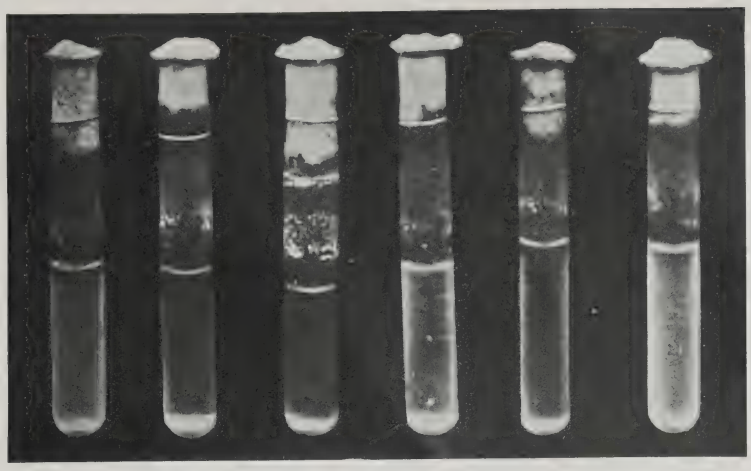

FIG. 4

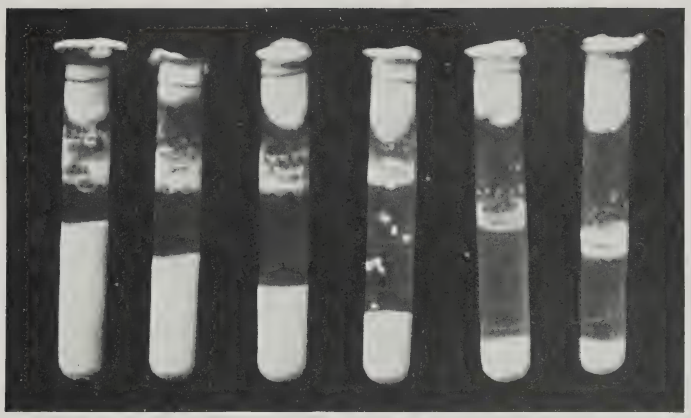

FIG. 5

FIG. 6

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EXPLANATION OF PLATE XXVII

Fig. 7. No decomposition of citrate. Voluminous white flocculent precipitate formed.

Fig. 8. Decomposition of citrate. Small heavy granular precipitate formed.

Fig. 9. To show solution of small granular precipitate seventeen days later decomposition of tartrate.

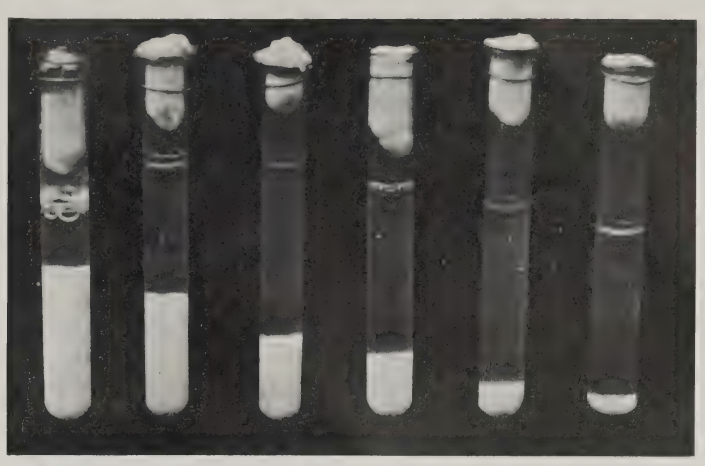

FIG. 7

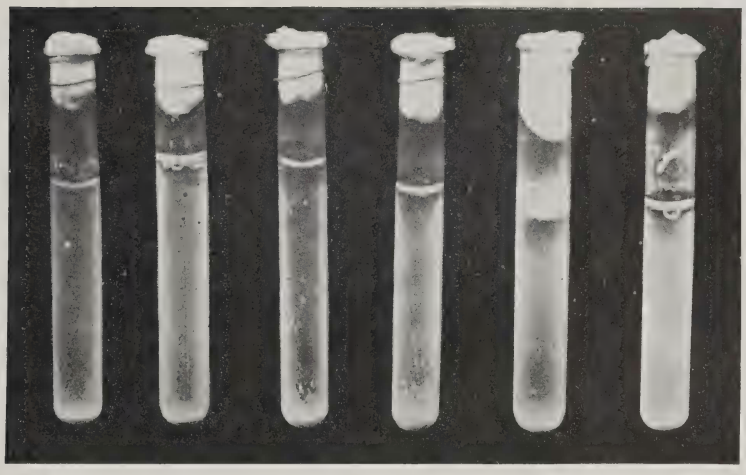

FIG. 8

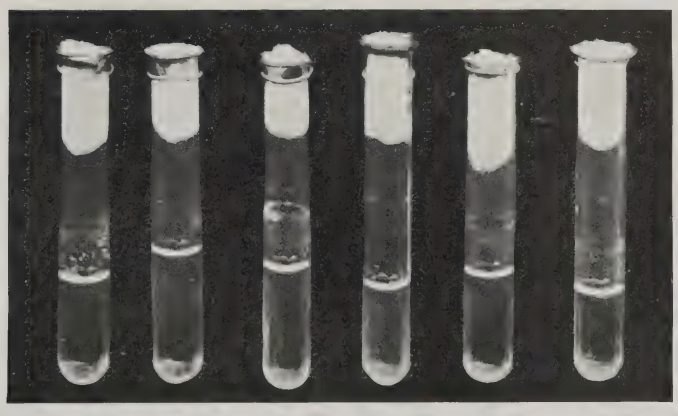

FIG. 9

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